'SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITIES OF PARACETAMOL DERIVATIVES: SPECTROPHOTOMETRIC VALIDATION FOR PHARMACOKINETICS STUDY'

Paracetamol (acetaminophen) was synthesized in 1878 by Morse (Morse et. al., 1878) and first used clinically by Von Mering in 1887 (Von et. al., 1893). However, during the period when phenacetin was popular, its use was dormant. The studies of Brodie and Axelrod (Brodie et. al., 1948) led to its "rediscovery" and marketing in the 1950s in the United States as an analgesic replacement for phenacetin, which was abandoned due to its nephrotoxicity. Unfounded concerns about acetaminophen safety delayed its widespread acceptance until the 1970s (Bertolini et. al., 2002). Acetaminophen is a non-steroidal anti-inflammatory drugs (NSAIDs) and mostly used as an analgesic and antipyretic drug. Acetaminophen is a weak anti-inflammatory action instead of it is class of NSAIDs, reports of a reduction in tissue swelling after dental surgery have been recorded (Skejelbred et. al., 1977, 1979). The main mechanism of action of paracetamol is to be the inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-3 in the brain and spinal cord explains the effectiveness of paracetamol in relieving pain and reducing fever without having unwanted gastrointestinal side effects (Chandrasekharan et. al. 2002). Paracetamol is a weak anti-inflammatory drug due to lack of prostaglandin inhibition peripherally in the body (Flower et. al., 1972). However, several reports stated that large overdoses of paracetamol can produce a fulminant hepatic and renal tubular necrosis due to the formation of the toxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) (Boyed et. al., 1971, Vermeulen et. al., 1990). Therefore, there has been a concerned search for the discovery and development of newer pharmacological active paracetamol derivatives.

The development of new drugs is one of the fundamental goals in medicinal chemistry. Researchers are always interested to synthesize new molecules from old or pre-

existing molecules and screen their pharmacological parameters. The pharmacological active molecule is very beneficial for the exploration of a new class of drug molecule. Pharmacological parameters are widely used for the therapeutic regimen setting and for the clinical study. Literature surveyed revealed that various pharmacological active paracetamol derivatives have been synthesized, including aceyl-ether derivatives (Silva et. al., 2011) and 3-nitroparacetamol (Al-Swayeh et. al., 2000) has been shown potential anti-inflammatory activity. 4-(2, 3-epoxy-propyl) acetaminophen (Profire et. al., 2010) has been shown a potential bronchodilatator effect. 2-(2-carboxyphenylsulfanyl)-N-(4-substituted phenyl) acetanilide derivatives (Ozkay et. al., 2011) have more analgesic potential than paracetamol. Varying substituent is a common method for drug design in medicinal chemistry. We aimed to synthesize new condensed paracetamol derivative and to test the antimicrobial and pharmacological activities by *in-vivo* and *in-vitro* tests.

The simple, efficient, sensitive, accurate and economical analytical technique for quantification of such newly synthesized derivatives is needed in pharmaceutical pasture. The development of novel techniques is used for the estimation of drug in different *in-vitro* and *in-vivo* pharmacological parameters such as, in pharmacokinetics, pharmacodynamic and bioequivalence study. A number of methods have been reported for the determination of paracetamol, such as a flow injection method (Erk et. al., 2001), liquid chromatography (Alkharfy et. al., 2001), capillary electrophoresis (Ruiz et. al., 2005), chemiluminescence (Ruengsitagoon et. al., 2006), electrochemical techniques (Kang et. al., 2010) and spectrophotometric methods. Spectrophotometric methods are based on nitration, oxidation and hydrolysis of p-aminophenol followed by diazotization and phenolic coupling. The determination of paracetamol in biological fluids (blood, plasma, urine) is mainly depended on HPLC techniques (West, 1981), electrochemical method (Lu et. al., 2012) and spectrofluorimetry (Vilchez et. al., 1995), while there are very few spectrophotometric

methods (Shihana et. al., 2010, Sebben et. al., 2010) in which paracetamol is determined in biological fluids. Different methods are used for kinetic study of the paracetamol, such as, bioavaibility data of paracetamol was determined by spectrophotometrically using saliva samples (Issa et. al., 2008).

In recent years, the throughput of the drug discovery process has improved because of the implementation of high-throughput in pharmacokinetics (PK) parameters such as, Absorption, Distribution, Metabolism and Elimination. Hundreds of compounds can be screened in vitro per week, providing scientists with a wealth of data. In vivo pharmacokinetic studies are included in various steps, study protocol preparation, animal dosing and sample processing and analysis (analytical portion), PK regression and data reporting. Paracetamol is well absorbed from the proximal small bowel and is not subject to significant first pass metabolism in the liver, with oral bioavaibility estimated at between 63-89% of adults (Oscier et. al., 2009, Rawlins et. al., 1977). Paracetamol is not significantly bound to plasma proteins, and has a volume of distribution of 0.7-1 L/kg. Maximal analgesic and antipyretic activity occurs 1-2 h after peak plasma levels (Oscier et. al., 2009, Ward et. al., 1999) and the time to achieve this varies with the route of administration. Peak plasma concentration (Cmax) is achieved approximately at 45 min (Oscier et. al., 2009). Metabolism of paracetamol occurs primarily in the liver, while elimination occurs almost entirely through the kidney. Following absorption of therapeutic doses, approximately 90% is metabolized by glucuronidation and sulphation to form non-toxic metabolites, which are excreted in the urine. Paracetamol, like many other analgesics, has a short half-life around 2-3 hours, which necessitates frequent dosing (Coulthard et. al., 2001). In the UK the recommended regimen is 500-1000 mg every 4-6 hours. However, it would be advantageous if the duration of action were longer so that fewer daily doses could maintain therapeutic plasma levels. This would improve patient convenience and compliance and be of benefit to the patient at night-time

(Coulthard et. al., 2001).

Preliminary studies provide interesting results and therefore detailed study was taken up for the synthesis of paracetamol derivatives and its pharmacological and pharmacokinetics studies.

Objective of work:

The present study is envisaged to synthesize new paracetamol derivatives, which are more effective potency than paracetamol drug. The acute oral toxicity study is also revealed new effective dose of the synthesized derivatives. The synthesized paracetamol derivatives show analgesic, antipyretic, anti inflammatory, antibacterial and antifungal activities. New spectrophotometric methods are also developed for the estimation of synthesized compounds in biological fluids. In vitro drug release profile study is carried out using Franz diffusion cell. Pharmacokinetics study is performed by using animal model and examines various parameters.

THE THESIS IS DIVIDED INTO SIX CHAPTERS

The aim of this work to synthesize new paracetamol derivatives, which are having more potential activities than the present paracetamol drug. The simple, accurate and cost-effective spectrophotometric methods are also developed for the estimation of synthesized compounds in biological fluids. In-vitro drug release study, pharmacological study and pharmacokinetic study of the synthesized derivatives are covered in this research work.

Chapter I: Introduction and Literature Review

This chapter comprises of the information on the scenario of paracetamol drug and lack of pharmacological activities potency. Literature studies revealed that pharmacological activity, toxicity study and lack of anti-inflammatory activity of paracetamol. Current

references along with the previous paracetamol derivatives have been more effective pharmacological activities than the presence paracetamol drug. Various spectrophotometric methods, which are used for biological fluids and pharmacokinetics study have been also reported. The objective and scope of the present work are also included.

Chapter II: Materials and method

The materials and synthesis method used during the whole research work are reported in this chapter.

Chapter III: Spectral characterization

The synthesized compounds are characterized by different spectral techniques (UV, FTIR, ^{1}H NMR, LCMS and Elemental analysis) and the physical parameters, such as %yield, melting point, solubility, TLC and pka value etc. are summarized in this chapter.

Chapter IV: Spectrophotometric validation method

This chapter describes the spectrophotometric validation method and quantitative determination of synthesized compound in biological fluids (blood and urine).

Chapter V: Pharmacological activities

The synthesized compounds have been shown various pharmacological activities (antibacterial, antifungal, analgesic, antipyretic and anti-inflammatory). Well plate method was used for the antibacterial and antifungal activities of the synthesized compounds. OECD guideline 423 was used for the evaluation of acute oral toxicity of the synthesized compounds. The analgesic study was carried out by Hot plate and Tail Immersion method; while antipyretic and anti-inflammatory activities were performed by Yeast induce Pyrexia and Carrageenan-induced paw oedema method, respectively. In-vitro drug release study was performed by using a Franz diffusion cell and the cellophane membrane (Cellulose acetate

membrane). The pharmacokinetic studies were carried out using Wistar albino rat and various parameters were measured by spectrophotometrically in blood and urine sample.

Chapter VI Results and Discussion

The structure of synthesized compounds was confirmed by FT-IR, ^{1}H NMR, LC-MS and elemental analysis. The synthesized compounds have shown excellent biological and pharmacological potencies than the present paracetamol drug. The synthesized compounds have been screened for antibacterial and antifungal activities and gives better results than the standard drug. Acute oral toxicity of the synthesized compounds was performed by using OECD guideline 423 and calculates the effective dose (ED_{50}) for the synthesized compounds. The results of analgesic and antipyretic activities revealed that the synthesized compounds are shown moderate to excellent potency than the paracetamol. Synthesized paracetamol derivatives of paracetamol are shown moderate to excellent anti-inflammatory activity than that of the standard diclofenac sodium drug. In vitro release studies for the synthesized compounds have been also evaluated. Pharmacokinetic studies were performed by an animal compartmental model method using blood and urine sample of the rats and calculates the different pharmacokinetic parameters such as, Tmax, Cmax, Kel, $T_{1/2}$, $(AUMC)_{0-\infty}$, $(AUC)_{0-\infty}$ and MRT, maximum rate of excretion (Rmax), Area under the rate of excretion versus midpoint of time interval curve for 0-84 hr and up to infinity [$(AURC)_{0-t}$ and $(AURC)_{0-\infty}$], cumulative amount of unchanged drug in urine (Ae), fraction of unchanged drug excreted (f) are discuss in this chapter.

Conclusion

The results and discussion is followed by the concluding remarks derived from this research work.

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